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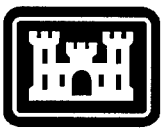

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Using Stable Isotopes of Carbon and Nitrogen as In-Situ Tracers for Monitoring the Natural Attenuation of Explosives

Paul H. Miyares, C. Michael Reynolds, Judith C. Pennington,
Richard B. Coffin, Thomas F. Jenkins, and Luis Cifuentes

December 1999

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Thank you.

Lisa

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14. ABSTRACT

The use of carbon and nitrogen stable isotope measurements from TNT was examined as a possible tool for monitoring the natural attenuation of TNT. Incubation studies of spiked soil samples were conducted. The concentration of TNT and the δel (δ) values for ^{13}C and ^{15}N of the soil and of TNT were measured with time. Results indicated that no measureable changes in the $\delta^{13}C$ or $\delta^{15}N$ value of the soil or $\delta^{13}C$ values for TNT could be detected as the concentration of TNT decreased. Measureable changes in the $\delta^{15}N$ value of TNT with changing TNT concentration suggested a possible monitoring route. Field studies using both soil and groundwater samples support the laboratory finding that changes in the $\delta^{15}N$ for TNT extracted from groundwater could be detected in samples collected at different points along transects at the Louisiana Army Ammunition Plant and that no measurable change in the $\delta^{13}C$ values for soil or TNT could be seen.

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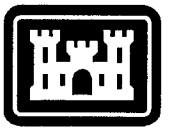

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PREFACE

This report was prepared by Dr. Paul H. Miyares and Dr. C. Michael Reynolds, Geochemical Sciences Division, Cold Regions Research and Engineering Laboratory (CRREL), and Judith C. Pennington, Environmental Processes and Effects Division, Environmental Laboratory, U.S. Army Engineer Research and Development Center, with technical contributions from Richard B. Coffin, Environmental Quality Sciences, Naval Research Laboratory, Dr. Thomas F. Jenkins, Geological Sciences Division, CRREL, and Luis Cifuentes, Department of Oceanography, Texas A&M University. Funding for this research was provided by the Strategic Environmental Research and Development Program (SERDP).

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CONTENTS

ILLUSTRATIONS

Figure

TABLES

Using Stable Isotopes of Carbon and Nitrogen as In-Situ Tracers for Monitoring the Natural Attenuation of Explosives

PAUL H. MIYARES, C. MICHAEL REYNOLDS, JUDITH C. PENNINGTON,
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INTRODUCTION

Background

One of the most serious environmental problems facing the U.S. Army is the contamination of the soil and groundwater with secondary explosives at many of its installations. Explosives contamination is the result of the manufacture of explosives, production of ordnance, and the disposal of off-specification and out-of-date material. Contaminants include the explosives, manufacturing impurities and by-products, and environmental degradation and transformation products (Walsh et al. 1993). One of the unique characteristics of these compounds is their mobility through soil, resulting in contaminated groundwater plumes, often miles in length (Kayser and Burlinson 1982, Pugh 1982, Rosenblatt 1986, Maskarinec et al. 1986, Spaulding and Fulton 1988).

The remediation efforts at these sites are currently twofold: source removal and groundwater treatment. The soils at the contamination sources are excavated, then either incinerated or treated by composting or anaerobic slurry digestion. The groundwater plumes are remediated almost exclusively using pump and treat systems. Although a number of research efforts are in progress to develop innovative treatment schemes, the principal method is passing the water through activated carbon. Remediation by pump and treat is slow, requiring years and perhaps decades to reach target concentrations. It is also expensive. With many new remediation efforts starting and the ever rising cost of energy, the price of pump and treat remediation for the Army is significant and increasing.

Natural attenuation has been proposed as an alternative remedial action at sites contaminated with explosives (Watson 1995). Precedence for this proposal was set by the widely implemented protocol for natural attenuation of fuels developed by the Air Force Center for Environmental Excellence (AFCEE) (Weidermeier 1994). Natural attenuation is defined as allowing the natural physical, chemical, and biological processes at a given site to reduce the levels of contaminants in the environment enough that they are no longer considered a health threat. Attenuation may occur through mineralization, transformation, or fixation (adsorption or chemical binding). Natural attenuation has the advantages of being nonintrusive and significantly less expensive than the currently used pump and treat systems, including monitoring.

Many organic compounds attenuate naturally in soils and sediments by microbiological mineralization to CO_2, biotic and abiotic transformation to other compounds, irreversible sorption, incorporation by chemically bonding into organic macromolecules such as humic and fulvic acids, transport, and volatilization (Marvin-Sillema and deBont 1994, McGrath 1995, Pennington et al. 1995, Pennington 1996). These processes are not necessarily exclusive and can occur both simultaneously and sequentially. Understanding the relative contribution of these cycles to the reduction of the lev-

els of specific compounds is a key for determining the fate of contaminants of interest (COI) in an environment. The conditions of the system, such as temperature, moisture, COI concentration, available carbon, chemistry, pH, and Eh, that exist during the process can affect the dominant remediation pathway by governing the rates of individual biological, geochemical, and physical processes. Not all transformation processes create more benign compounds. Measuring the direction and rates of competing processes under different conditions is necessary for predicting the fate of compounds undergoing natural attenuation, and allows one to set a time-scale for remediation of an ecosystem and to prioritize remedial efforts.

Rates for attenuation of a COI can be estimated in laboratory studies conducted under representative field conditions, but there remains a need to confirm the rates with field data. One approach is through modeling and subsequent model validation. This approach typically uses changes in concentrations of readily measured reactants, final products, or both, to evaluate the success of describing intermediate processes. While laboratory analysis indicates the potential pathways for attenuation of a COI, in-situ analysis is required to provide confirmation of pathways and rates of disappearance. For assurance that these processes are in fact occurring, it is desirable to use field monitoring to confirm both laboratory tests and model predictions.

Degradation pathways for 2,4,6-trinitrotoluene (TNT) have been documented previously (McCormick et al. 1976). In soil systems, TNT is believed to be reduced to several isomeric aminodinitrotoluenes, then sequentially to diaminonitrotoluenes. Recent evidence indicates that these mono and diamino compounds can form covalent bonds to natural organic matter (Thorn 1995, Thorne and Leggett 1998) and thus be humified. The influences of moisture, temperature, and soil organic carbon on the fate of processes affecting TNT can be evaluated in the laboratory using ^{14}C TNT to measure respiration or humification or assimilation, and the resulting rates can be used for making field predictions. Two potential limitations arise from this approach: 1) ^{14}C labeled compounds generally can not be used for field confirmation, and 2) there may be some isotopic fractionation during the process (Galimov 1981). An innovative alternative that has received limited investigation is using stable isotopes, ^{13}C and ^{15}N, as naturally occurring tracers (Van de Velde et al. 1995).

Theory

Carbon is primarily composed of two isotopes, ^{12}C, which accounts for 98.89% of all carbon, and ^{13}C, which accounts for approximately 1.11% (Craig 1957, Faure 1986, Hoefs 1987). The relative abundance of ^{13}C is expressed as $\delta^{13}C$ values, which represent the ^{13}C to ^{12}C ratio relative to a standard ratio from the Pee Dee Belemnite (PDB) Formation, Upper Cretaceous, South Carolina. Nitrogen is also composed of two isotopes, 99.6% of which is ^{14}N and 0.4% is ^{15}N (Watson 1985). The relative abundance of ^{15}N in a sample is determined vs. that in atmospheric nitrogen.

Isotope ratios are reported as a difference, rather than absolute concentrations, because this provides a more useful measure in the description of isotope behavior, as well as being more precisely measured (Friedman and O'Neil 1977, Lajtha and Michener 1994). These difference values—δ or "del"—are calculated using eq 1 and are expressed in parts per thousand, often referred to as "per mil" and generally denoted as $^{0}/_{00}$. Compounds that are depleted of the heavy isotope relative to the standard have negative δ values, while compounds that are enriched in the heavy isotope have positive δ values.

$$\delta = ([R/R_s) - 1]) \times 1000 \quad (1)$$

where R is the absolute isotope ratio in the sample, and R_s is the absolute isotope ratio in the standard.

Previous uses

Stable isotope analysis has been used to address a broad range of topics in C, N, O, H, S, and Cl cycling. The natural differentiation in stable isotope values, such as $\delta^{13}C$ and $\delta^{15}N$ levels, can be used as tracers for following the long-term dynamics of natural systems (Balesdent et al. 1987, 1988). In the oil industry, petroleum compounds have been linked to the source rock formation from which they have migrated by relating the $\delta^{13}C$ values of specific petroleum fractions with those in the kerogen (organic) fraction of the source rock (Stahl 1980). In soil science, soil organic matter turnover rates have been estimated by comparing changes in $\delta^{13}C$ values for different soil organic fractions (O'Brien and Stout 1978, Boutton et al. 1980), and $\delta^{13}C$ has been used for determining sources of CO_2 evolved from soil (Reardon et al. 1979). Stable isotope ratios have also been used to estimate long term changes in plants and soils (Freeman and Hayes 1992).

Stable isotope analysis has also been employed

to trace short term cycling of organic matter through food chains in ecosystems (Peterson et al. 1985, Peterson and Fry 1987). It has been used to study microbial roles in elemental cycling (Blair et al. 1985; Coffin 1989; Coffin et al. 1990, 1994; Coffin and Cifuentes 1993), to identify carbon sources that support food chains in complex aquatic ecosystems (Coffin 1989; Coffin et al. 1990, 1994; Coffin and Cifuentes 1993; Cifuentes et al. 1996a,b), and to analyze nitrogen cycling (Peterson and Fry 1987; Hoch et al. 1992, 1996). As a result of technological limits, preliminary studies have focused on the major component of elemental cycles in complex pools with numerous sources. Recently, with the attachment of gas chromatographs (GC) inlet to the isotope ratio mass spectrometers (IRMS), research has moved to studying the cycling of specific compounds in ecosystems (O'Malley et al. 1994; Trust et al., in press) and to identifying individual compounds as biomarkers to determine microbial roles in biogeochemical cycles (Pelz et al. 1998).

Recent developments allow stable isotope analysis to be used for evaluating remedial efforts in environments that are contaminated with petroleum hydrocarbons. This technology provides us with the capability to identify sources of contaminants in ecosystems (O'Malley et al. 1994; Kelley et al., in press). In-situ degradation of organic contaminants is studied using ^{13}C analysis of CO_2 evolving from soil (Cifuentes et al. 1996a,b; Van de Velde et al. 1995). Finally, specific biomarkers have been used to evaluate the bacterial assimilation of contaminants (Pelz et al. 1998).

It should be feasible to use a similar technique for estimating TNT attenuation rates in subsurface soils. Since TNT was manufactured from petroleum-based compounds, the carbon ring in TNT should retain depleted $\delta^{13}C$ values similar to those that are measured in petroleum compounds. A primary fate of TNT in many environments appears to be bonding to or incorporation with the soil organic fraction rather than mineralization (Pennington et al. 1995, Pennington 1996); therefore, significant evolution of $^{13}CO_2$ derived from TNT is not expected.

Rationale

TNT in a contaminated area undergoes two major processes: reaction with the soil through which it moves, and transport with the groundwater. These processes simultaneously influence the fate of TNT in soil and groundwater systems, but separation of reaction and transport processes may be possible in the laboratory. If so, the reaction and transport components could be more mechanistically included in models.

The stable isotopes ^{13}C and ^{15}N were used as tracers to track the processes that take place during TNT degradation. Dual tracers allow measurement of attenuation whether the process is governed by nitro/amino functionality or aromatic ring/toluene functionality, or both. The following two strategies were used: measuring carbon-based changes in the soil, and nitrogen-based changes in solution.

Soil carbon

Incorporation of TNT into soil humic material or covalent bonding of amino-daughter products of TNT with the same may result in a change in the $\delta^{13}C$ of the soil during or after binding. Measuring differences between $\delta^{13}C$ values in TNT and in soil is complicated by the following three factors:

- Scant data are available on $\delta^{13}C$ values for TNT from different sources and soil $\delta^{13}C$ values vary with parent material and biological–chemical processes.
- The $\delta^{13}C$ values may change very slowly under field conditions.
- The amount of TNT-derived carbon binding to soil, and hence the change in $\delta^{13}C$ of the soil carbon after attenuation, may be too small to measure relative to the native carbon levels in the soil. Low-carbon soils would have less "masking" ability than high-carbon soils and would, in theory, require less difference in soil and TNT $\delta^{13}C$ values to detect changes. Deep aquifer soils, however, may contain very low masses of organic carbon, resulting in detection limit difficulties.

Solution nitrogen

The amino reduction products of TNT are believed to bond directly through the amino functional group to the soil humic material (Thorn 1995). Therefore, one or two of the nitro groups on the TNT must first be reduced to amino groups. The reduction of nitro groups, whether chemically or biologically, may result in isotopic fractionation because of reaction-rate differences between ^{15}N and ^{14}N. If so, a relatively greater percentage of ^{14}N should be reduced and involved in reactions with either humic material or clay. This process would leave relatively more ^{15}N in the TNT remaining in the groundwater. In effect, the $\delta^{15}N$ values of TNT in groundwater would evolve

toward the heavy isotope of nitrogen. Two advantages to measuring the $\delta^{15}N$ values of TNT in the groundwater are: 1) groundwater samples are easier to obtain than subsurface soils samples, and 2) groundwater is a well-mixed system relative to the soil through which it passes. A disadvantage is that the concentrations of TNT may be too low to obtain reliable $\delta^{15}N$ values.

Regardless of the mechanism, changes in the $\delta^{15}N$ values of TNT in groundwater toward heavier N would indicate that some form of attenuation is ongoing.

Objective

The objective of this work was to evaluate the feasibility of using ^{13}C and ^{15}N stable isotope analyses as an analytical tool for monitoring natural attenuation at explosives contaminated sites. Laboratory studies were conducted to determine if changes in the $\delta^{13}C$ and $\delta^{15}N$ values for TNT in solution and the $\delta^{13}C$ values for the soil carbon could be measured as TNT proceeded through its degradation and attenuation processes. Field studies were conducted using contaminated groundwater from Louisiana Army Ammunition Plant (LAAP) to determine whether changes in the $\delta^{13}C$ and $\delta^{15}N$ values for TNT in groundwater could be measured as a function of time or distance as the TNT plume migrated through the soil. Field studies were conducted using soil samples from LAAP to determine if the $\delta^{13}C$ values for the soil carbon changed over time as a result of exposure to and attenuation of TNT.

METHODS AND MATERIALS

Instrumental analyses

HPLC analysis

Sample extracts for RP-HPLC (reversed phase high-performance liquid chromatography) analysis were diluted 1:4 (v/v) with water and filtered through a Millex SR 0.5-µm filter (Millipore Corp., Bedford, Massachusetts). The chromatographic system consisted of a Spectra Physics 8800 programmable pump (Spectra Physics, Inc., San Jose, California) operated in isocratic mode, a Spectra Physics Spectra 100 variable wavelength detector set to 254 nm, and a Dynatech LC-241 autosampler (Dynatech Corporation, Baton Rouge, Louisiana). The analytes were separated on an LC-8 (150 × 3.9 mm) column (Waters Corp., Milford, Massachusetts), eluted with a binary eluant of isopropanol/water (15:85, v/v) at a flow rate of 1.4 mL/min. The column temperature was maintained at 28°C with a column oven.

GC-IRMS analysis

Stable carbon and nitrogen isotope analysis of nitroaromatics in solution was conducted with a Varian Star 3400 CX gas chromatograph (GC) (Varian, Harbor City, California), equipped with a Finnigan Magnum ion trap mass spectrometer (ITMS) and a Finnigan Delta-S isotope ratio mass spectrometer (IRMS) (Finnigan Corporation, San Jose, California) (GC/ITMS/IRMS). The analytes were separated on an SPB-1 fused silica capillary column (15 m × 0.32 mm i.d., 0.25-µm film thickness) using a temperature program of 100 to 250°C at 8.3°C/min. The effluent from the column was split, with 10% going to the ITMS for peak identification and the remainder to the IRMS where the analytes were combusted in line at 940°C to CO_2 and N_2 for isotope analysis. For $\delta^{15}N$ analysis, the capillary transfer line was submerged in liquid nitrogen to remove carbon gases from the N_2. The standard for nitrogen was atmospheric N_2 and the standard for carbon was Pee Dee Belemnite. The detection limits were 1 µg C and 3 µg N, and the precision was ±0.2‰ for $\delta^{15}N$ and ±0.1‰ for $\delta^{13}C$.

Dual inlet IRMS analysis

Soil samples were analyzed isotopically by a modified Dumas combustion method that converts organic carbon and organic nitrogen to CO_2 and N_2 for mass spectral analysis (Macko 1981). Soil samples were placed in quartz tubes with Cu and CuO, evacuated, and sealed. The quartz tubes were then heated to 850°C at a rate of 450°C/hr, kept at 850°C for 2 hours, and cooled to room temperature at a rate of 600°C/hr. The slow cooling cycle ensured that any oxides of nitrogen were decomposed to N_2. The CO_2 was separated from N_2 by cryogenic distillation. The N_2 gas was then analyzed on a Nuclide 3-60-RMS. In turn, CO_2 gas was analyzed on a Finnigan MAT 252 IRMS (Finnigan Corporation). The standard for nitrogen was atmospheric N_2 and the standard for carbon was Pee Dee Belemnite. The detection limits were 1 µg C and 3 µg N, and the precision was ±0.2‰ for $\delta^{15}N$ and ±0.1‰ for $\delta^{13}C$.

Calibration experiment

A series of standard solutions of standard analytical reference material (SARM) grade TNT (Army Environmental Center [AEC], Aberdeen Proving Ground, Maryland) were prepared in acetonitrile (AcN), with concentrations ranging from

2.0 to 1000 mg/L. Each standard was analyzed by GC-IRMS and the $\delta^{13}C$ value for TNT was determined. The relationship between concentration and the $\delta^{13}C$ value for TNT was computed and a minimum concentration threshold was determined where reliable data could be obtained.

TNT solutions

An aqueous solution of SARM grade TNT (AEC) was prepared by placing 250 mg of TNT into 4 L of MilliQ grade water and stirring for 3 days. The solution was filtered to remove any undesolved TNT then analyzed by HPLC. The resulting concentration was determined to be 49.5 mg/L. This solution was used to spike the samples in incubation study 1.

A second aqueous solution of TNT was prepared by placing 3 g of technical grade TNT (Eastman Kodak, Rochester, New York) into 20 L of tap water. The solution was stirred for 10 days, then filtered, and analyzed by HPLC. The resulting concentration was determined to be 101 mg/L. This solution was used to spike samples in incubation study 2.

TNT source comparison

Samples of TNT were obtained from five different sources: SARM grade (AEC), technical grade (Eastman Kodak), and two U.S. Military grade samples (Picatinny Arsenal, New Jersey, and Sandia National Laboratory, Albuquerque, New Mexico) and Croatian military grade removed from a PMA1A antipersonnel landmine. Solutions of each TNT were prepared at a concentration of 1 g/L in AcN. Each solution was analyzed by GC-IRMS for $\delta^{13}C$ and $\delta^{15}N$ values for TNT.

Laboratory studies

Incubation study 1: Isotope ratio of TNT-amended soil over time

Two soils were chosen for the incubation study, one from Charlton, New Hampshire, and the other from Louisiana AAP. The Charlton soil was a sandy loam with 1.3% total organic carbon. The LAAP soil was primarily sand with a total organic carbon of 0.012%. Both soils were oven dried at 105°C then sieved through a no. 20 sieve (0.84 mm). Samples were prepared by placing 25 g of soil into a 50-mL test tube (50 samples of each soil were prepared). A 2.0-mL aliquot of tap water was added to each sample to rewet the soil and allow microbiological activity to restart. The samples incubated in the dark at room temperature for 3 days. A 4.0-mL aliquot of aqueous TNT (49.5-mg/L) solution was added to 35 of the 50 samples. A 4.0-mL aliquot of MilliQ grade water was added to the remaining 15 samples to be used as blanks. The samples were returned to the dark and incubation continued at room temperature (22 ± 2°C).

Sampling was done immediately after preparation (time zero), then at 1, 3, 7, 14, 21, and 28 days. For each sampling day, three replicates of each spiked soil were chosen at random. Blanks were sampled in triplicate on days 0, 3, 14, and 28. Each soil sample was quantitatively transferred from the incubation tube to an extraction thimble and extracted by Soxhlet for 24 hours with AcN at a rate of six cycles per hour. The final extract was diluted volumetrically to 250 mL. The soil was allowed to air dry overnight, giving the AcN time to evaporate, then oven-dried at 105°C overnight to remove any remaining water or solvent. Extracts were analyzed for concentration of TNT and its transformation products by HPLC-UV as described above and by GC-IRMS for $\delta^{13}C$ and $\delta^{15}N$ values for TNT. The dried soil was analyzed by dual inlet IRMS for $\delta^{13}C$ and $\delta^{15}N$ values of the soil.

Incubation study 2: Unlimited contaminant source, maximum loading of soil

A soil collected at Hanover, New Hampshire, was oven dried (105°C) and sieved as described above. Each slurry sample was prepared by placing 25 g of soil into 250-mL glass jars. A total of 48 samples was prepared. To each soil, 6.0 mL of tap water was added to rewet the soil. The samples were then allowed to incubate in the dark at room temperature for 3 days. A 100-mL aliquot of aqueous TNT solution (101 mg/L) was added to 39 of the 48 samples. To the remaining nine samples, 100 mL of tap water was added. The samples and blanks were shaken on a wrist action shaker for 20 minutes, then returned to the dark for incubation. Also prepared at this time were three control samples containing only 100 mL of the TNT spiking solution plus 6 mL of tap water (no soil).

The spiked soils were sampled at days 0, 7, 14, 21, 28, and 56. Blanks were sampled at days 0, 21, and 56. On each sampling day, all of the jars were shaken for 20 minutes on a wrist action shaker, then centrifuged for 45 minutes at 2000 rpm. Three samples were randomly selected and place to one side for analysis. (At every third sampling, three blanks were also selected.) For the remaining samples, the aqueous phase was decanted, weighed, then discarded. A fresh 100-mL aliquot of the technical grade TNT solution was then

added to each sample. The samples were again shaken for 20 minutes, then returned to the dark.

The samples selected for analysis were centrifuged for 45 minutes at 2000 rpm. The aqueous phase was poured off, weighed, then transferred to a brown glass jar. The aqueous phase was analyzed by HPLC as described above to determine the concentration of TNT and the 2-amino- and 4-amino-dinitrotoluene reduction products. Three subsamples of each soil phase were placed in aluminum tins and weighed. The subsamples were air dried for several days until a constant weight was achieved. The dried subsamples were extracted with AcN and the extracts analyzed by HPLC as described above to determine the concentration of the analytes of interest and by GC-IRMS for the $\delta^{13}C$ and $\delta^{15}N$ values for TNT. The remainder of the soil was extracted by Soxhlet extractors for 24 hours with AcN, dried, then analyzed by dual inlet IRMS for the $\delta^{13}C$ and $\delta^{15}N$ values.

Field studies

Isotope ratios across the contaminated plume at LAAP

Four transects were drawn across the LAAP site (Fig. 1) between wells in the source region of the TNT plume and wells along the leading edge of the plume. Table 1 describes the specifics of each transect. Six 1-L samples were collected from each of the six wells selected in April 1998 and then again in September 1998.

The TNT was extracted from the groundwater using Bond Elut EVN 200-mg/3-cm^3 solid phase extraction cartridges (Varian, Harbor City, California). Duplicate 1-L samples were extracted for the source region samples and duplicate 2-L samples were extracted for the leading edge samples. The groundwater samples were passed through the cartridges at a rate of approximately 10 mL/min. Because of the high TNT concentration of the samples, two cartridges were placed in series to prevent losses of analyte resulting from any breakthrough. The TNT was recovered from the car-

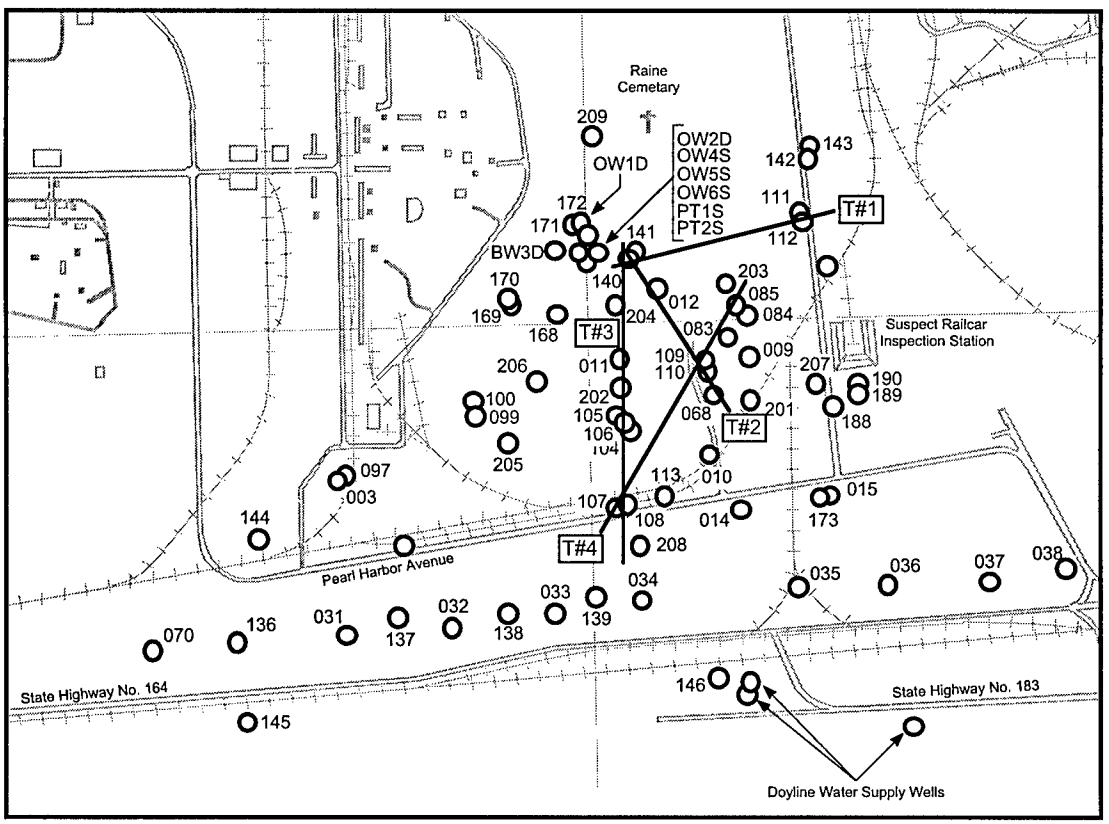

Figure 1. Site map and monitoring well locations at LAAP.

Table 1. Description of transects along which groundwater samples were collected.

Transect number	*Well in source region*	*Well at leading edge*	*Aquifer*
1	141	112	Lower
2	141	110	Lower
3	140	108	Upper
4	085	108	Upper

tridges with 5.0 mL of AcN and the concentrations determined by HPLC. The extracts were then analyzed by GC-IRMS to determine the $\delta^{13}C$ and $\delta^{15}N$ values for TNT.

Isotope ratio in soil at LAAP

Soil core samples were collected from five locations at LAAP using the Site Characterization and Analysis Penetrometer System (SCAPS) (Table 2). At each location, subsamples were obtained from 5 to 14 different depths, ranging from 1.5 to 20.5 m for determination of TNT concentration and stable isotope ratios for extractable TNT and soil carbon.

The soil samples were air dried, ground with a mortar and pestle, then passed through a no. 20 sieve. A 2.0-g subsample of each soil was extracted with AcN. The extract was analyzed by HPLC to determine the concentration of TNT and the monoamino-dinitrotoluenes. An additional subsample of each soil was washed twice with 10 mL of AcN for 18 hours with ultrasonication, air dried, then analyzed by dual inlet IRMS to determine the $\delta^{13}C$ and $\delta^{15}N$ values for the soil.

RESULTS AND DISCUSSION

Method development

This project's initial task was to develop sample preparation techniques that would allow isolation and preconcentration of TNT, but would not intro-

Table 2. Locations and depths from which the soil samples from LAAP were collected.

Location no.	*Hole no.*	*Depth (m)*
2	1	7.8
2	1	10.2
2	1	14.4
2	1	18.9
2	3	5.6
2	3	13.4
2	3	17.4
4	2	1.6
4	2	3.1
4	2	4.6
4	2	5.4
4	2	7.7
4	2	9.1
4	2	10.7
4	2	12.2
4	2	13.8
4	2	15.2
4	5	1.5
4	5	3.1
4	5	4.6
4	5	6.1
4	5	7.6
4	5	9.4
4	5	10.7
4	5	12.2
4	5	14.2
4	5	15.2
4	5	16.8
4	5	18.3
4	5	20.5
Location no.	*Hole no.*	*Depth (m)*
6	2	1.5
6	2	3.1
6	2	4.6
6	2	6.1
6	2	7.6
6	2	8.2
6	2	9.1
6	2	10.4
6	2	12.3
6	2	13.1
6	2	13.7
6	2	15.2
6	2	17.5
6	2	18.3
6	3	6.3
6	3	10.6
6	3	13.1
6	3	15.9
6	3	17.9

duce isotopic fractionation. Two methods had to be developed, one for soil samples and one for water samples. The goal of each method was exhaustive extraction with no isotopic fractionation, because if this occurred during sample preparation, it would confound the results.

For soil samples, the most efficient means of exhaustive extraction is the Soxhlet extractor. Although Soxhlet extractions are slow, this technique has the unique characteristic that the analytes are completely separated from the soil in the extractor because the solvent reservoir–collection vessel and sample holder are physically separated. In the case of ultrasonic extraction, the sample and extraction solvent are in the same vessel. Complete (100%) removal of the solvent for an ultrasonic extraction is not possible. Some residual solvent remains with the sample and this solvent will contain some of the analytes. With Soxhlet extraction, once the analytes are passed to the collection vessel, they cannot again come in contact with the soil. The results of an extraction kinetics experiment showed that a 24-hour extraction was required to obtain 99.9% recovery of TNT from field contaminated soil using AcN as the extraction solvent.

For extraction of TNT from water samples, solid phase extraction (SPE) was the method of choice. The groundwater was passed through an SPE cartridge at 10 mL/min and the analytes were recovered with 5 mL of AcN. The recovery for TNT was 99.8%.

Spiked soil and water samples were prepared and processed using the methods described above. The extracts from these samples were analyzed to determine the $\delta^{13}C$ and $\delta^{15}N$ values for TNT. These results were compared to the $\delta^{13}C$ and $\delta^{15}N$ values for TNT dissolved in solution at the same concentration as the extracts. Results for both techniques showed that there was no isotopic fractionation during the sample preparation processes (Table 3)

Table 3. Comparison of $\delta^{13}C$ and $\delta^{15}N$ values for TNT subjected to different sample preparation processes.

	$\delta^{13}C$	$\delta^{15}N$
Control solution	–28.2	3.41
SPE cartridge extract	–28.0	3.42
Soxhlet extract	–28.2	3.41

Calibration study

The concentration of TNT in moist soil samples is known to decrease with time under certain environmental conditions (Grant et al. 1993). Thus, the mass of TNT recovered over a time course experiment will decline as time passes. The objective of this calibration experiment was to determine if $\delta^{13}C$ values for TNT in solution varied as a function of concentration or mass injected into the analyzer. The results in Table 4 show that, above 4.0 mg/L (or 4.0 ng injected) in solution, the $\delta^{13}C$ values for TNT, although varied, are not greatly affected by the changing concentration. The result for the 1.96-µg/L (2.0-ng) standard shows a dramatic increase in the $\delta^{13}C$ value for TNT (Table 4). These data indicate that, below a concentration threshold of 4 mg/L, the $\delta^{13}C$ value for TNT takes on a concentration dependency. This dictates that all analyses require an injection mass of 4 ng or greater.

Analysis of TNT from multiple sources

TNT was obtained from several sources, providing SARM, technical grade, and three military grade samples. Solutions of each material were analyzed to determine the $\delta^{13}C$ and $\delta^{15}N$ values for TNT. The objective of this experiment was to establish a working range for these values using real material that may be present at a TNT-contaminated site. The results are presented in Table 5 and Figure 2.

There were significant differences in both the $\delta^{13}C$ and $\delta^{15}N$ values for TNT among the different sources. These differences can be attributed to the actual materials used to produce each TNT and the level of refinement for each. The SARM grade is noticeably the most different. It is also the most highly refined. For the military grade samples, the

Table 4. Concentration of TNT in solution versus $\delta^{13}C$ value for TNT.

Concentration of TNT (mg/L)	*Mass of TNT injected (ng)*	*$\delta^{13}C$ value for TNT (‰)*
1001	1000	–31.9
501	501	–31.2
250	250	–28.5
125	125	–29.6
62.5	62.5	–27.9
31.3	31.3	–26.2
15.6	15.6	–25.4
7.8	7.8	–29.4
3.9	3.9	–34.5
1.96	2.0	–17.2

Table 5. $\delta^{13}C$ and $\delta^{15}N$ values for different sources of TNT.

	$\delta^{13}C$	*Standard deviation*	$\delta^{15}N$	*Standard deviation*
SARM	–26.42	0.47	3.41	0.84
SNL	–22.21	0.21	9.64	0.89
PA	–23.36	0.14	3.58	0.57
Kodak	–22.69	0.30	2.58	0.54
Croatian	–23.04	0.22	–5.38	1.15

SARM = Standard Analytical Reference Material; SNL = U.S. military grade obtained from Sandia National Laboratory; PA = U.S. military grade obtained from Picatinny Arsenal; Kodak = technical laboratory grade from Eastman Kodak; Croatian = military grade from Croatia taken from a PMA1A antipersonnel land mine.

range for the $\delta^{13}C$ values is small, –23.0‰ (Croatian) to –22.2‰ (SNL). The range for the $\delta^{15}N$ values is much broader, –5.38‰ (Croatian) to 9.64‰ (SNL).

Laboratory studies

Incubation study 1: Isotope ratio of TNT-amended soil over time

For the Charlton soil, the concentration of TNT decreased rapidly over the first 7 days (Fig. 3). The concentration of the 2-amino- and 4-amino-dinitrotoluene (2-Am-DNT and 4-Am-DNT) reduction products increased during the first 7 days. After day 14, the concentrations of all of the analytes decreased with time. These concentration changes agree with previous results from the monitoring of TNT spiked onto wetted soils (Grant et al. 1993). The mass balance calculations indicated that recoverable TNT and transformation products decreased by 70% from the original mass of TNT in just 21 days (Fig. 4). A much smaller decrease in concentration of TNT and a small increase in the concentration of the amino-dinitrotoluenes was observed in the LAAP soil. The total loss of extractable TNT and transformation products was 37% over 77 days (Fig. 5). No significant change in the $\delta^{13}C$ value for the extractable TNT (Fig. 6 and 7) or for the $\delta^{13}C$ of the soil carbon was observed (Fig. 8).

The data above (Fig. 6 and 7) showed that, although the concentration of extractable TNT decreased during incubation, no changes could be detected in the $\delta^{13}C$ value for TNT. This was consistent with predicted results. A measurable change in the $\delta^{13}C$ value for the soil was sought as the TNT became irreversibly bound to the soil organic matter. Figure 8 showed that no change the in $\delta^{13}C$ value for the soil could be measured over the length of the experiment. Thorne (Thorne and Leggett 1998) reported that the highest percentage of TNT bound to organic matter that he had measured in finished compost was 0.2% (w/w). The total percent carbon in the soils used

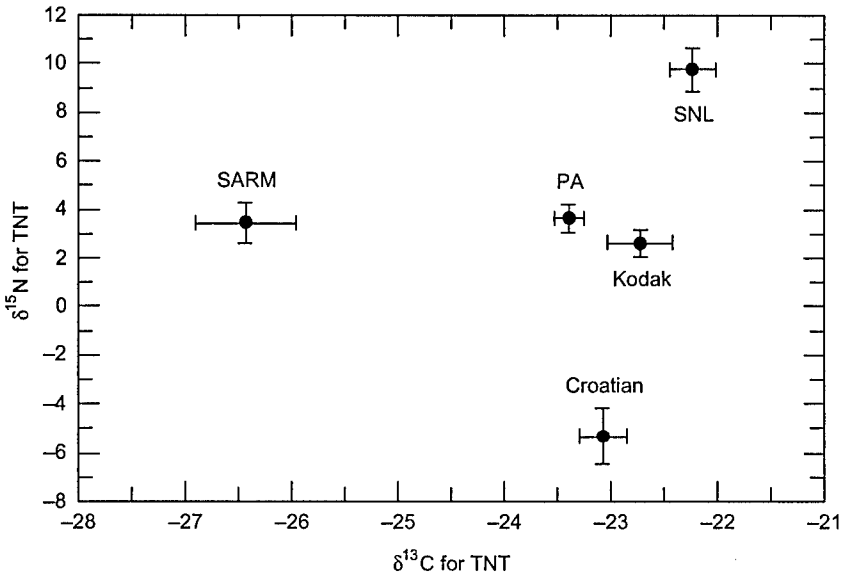

Figure 2. Plot of $\delta^{15}N$ vs. $\delta^{13}C$ for TNT from several different sources.

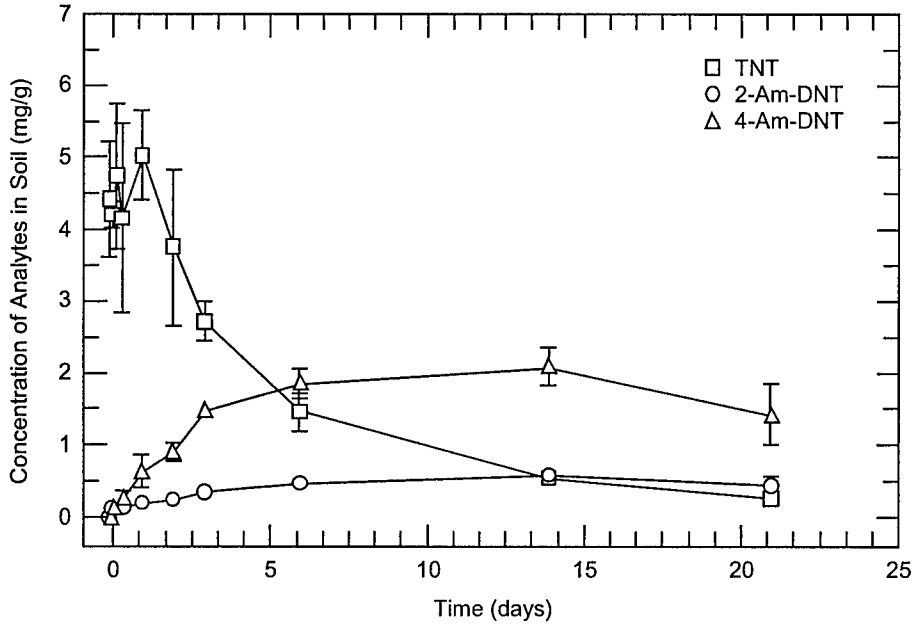

Figure 3. Concentration of TNT, 2ADNT, and 4ADNT over time for the experiment using Charlton soil.

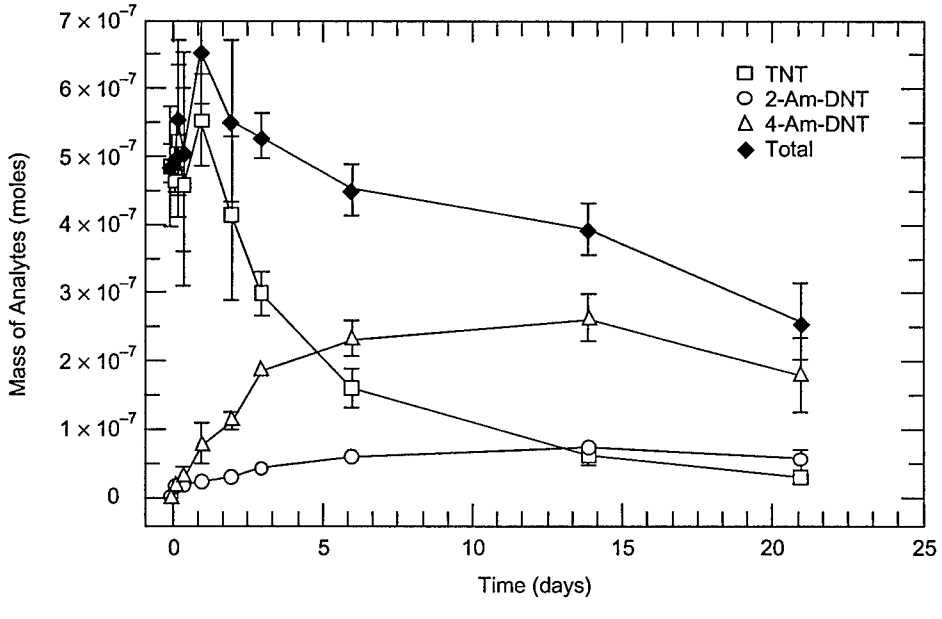

Figure 4. Mass of TNT, 2ADNT, and 4ADNT, and total mass recovered over time for the experiment using Charlton soil.

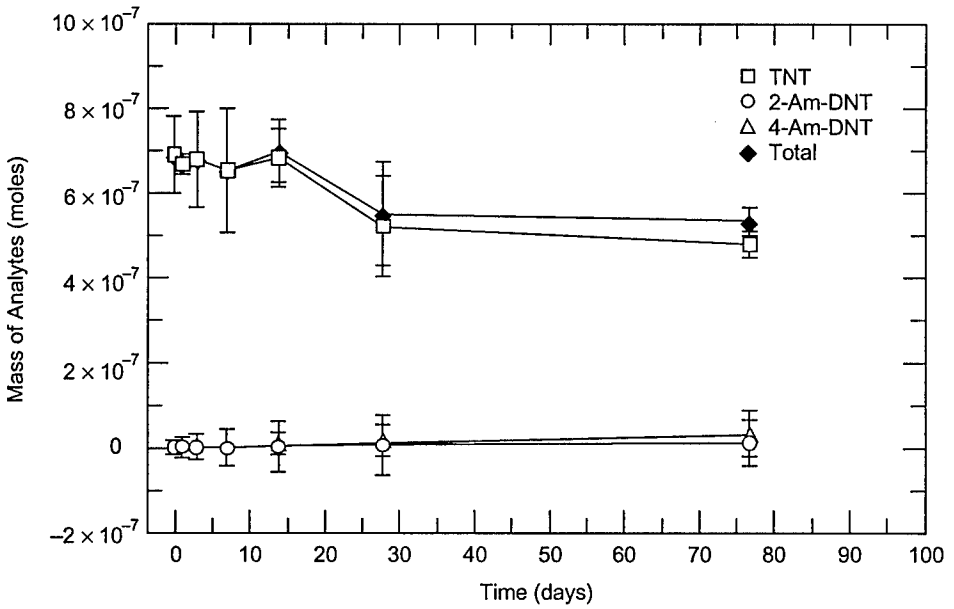

Figure 5. Mass of TNT, 2ADNT, and 4ADNT, and total mass recovered over time for the experiment using LAAP soil.

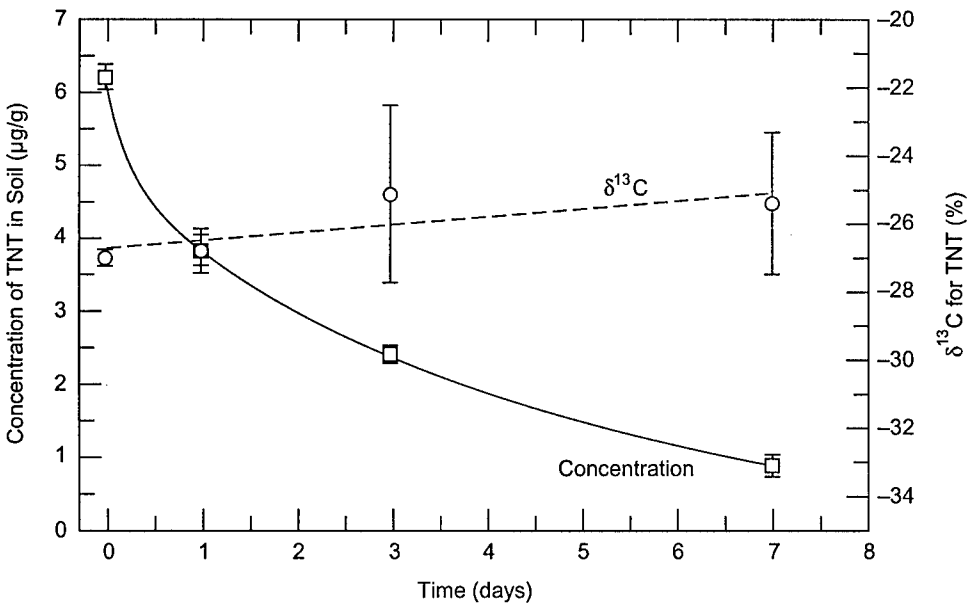

Figure 6. Concentration of TNT extracted from the soil and the $\delta^{13}C$ value for extractable TNT over time for the experiment using Charlton soil.

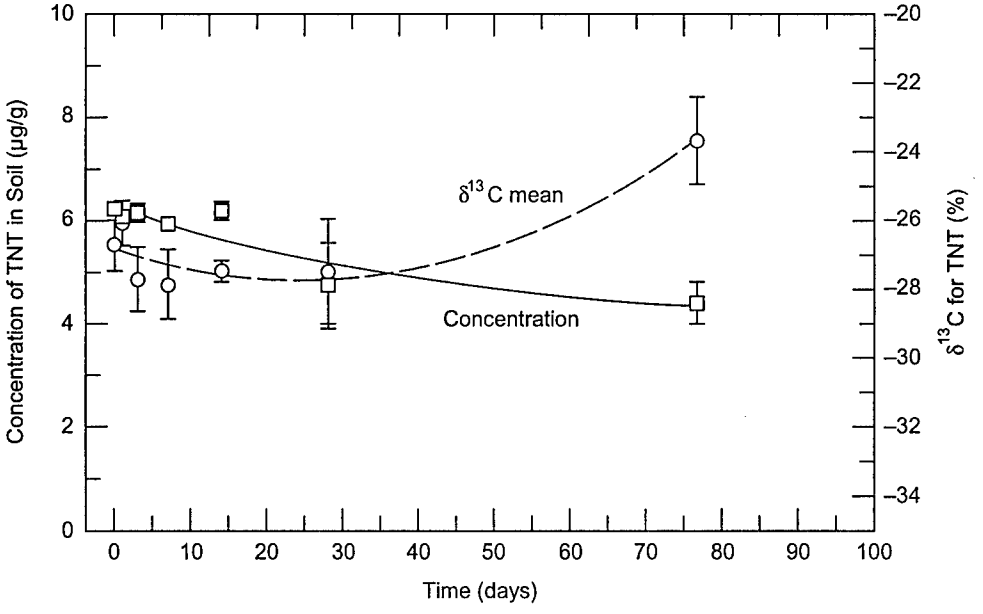

Figure 7. Concentration of TNT extracted from the soil and the $\delta^{13}C$ value for extractable TNT over time for the experiment using LAAP soil.

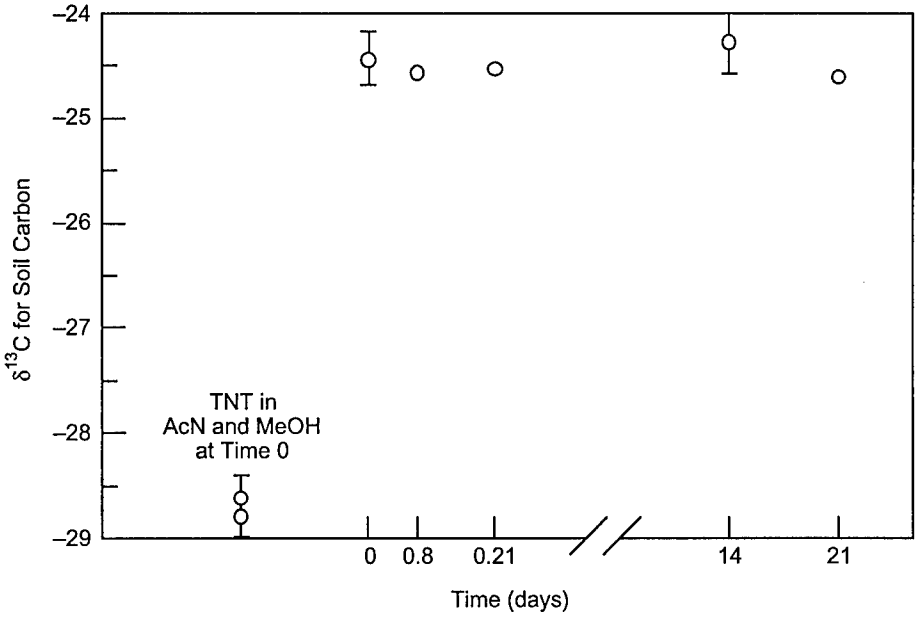

Figure 8. Change in $\delta^{13}C$ of the soil carbon in the Charlton soil over time.

Table 6. Concentration of TNT, 2ADNT, and 4ADNT in the aqueous and soil phases in incubation study 2.

	Days					
	0	*7*	*14*	*21*	*28*	*56*
TNT in aqueous phase (mg/L)	93.38	81.64	90.72	97.00	95.80	94.34
2ADNT in aqueous phase (mg/L)	0.000	0.095	0.207	0.141	0.119	0.216
4ADNT in aqueous phase (mg/L)	0.00	0.354	0.722	0.417	0.283	0.478
TNT in soil (μg/g)	0.00	6.35	24.82	30.40	25.81	24.47
2ADNT in soil (μg/g)	0.00	0.236	0.114	0.290	0.091	0.095
4ADNT in soil (μg/g)	0.00	1.467	0.814	0.661	0.424	0.428

in these studies ranged from 0.012% for LAAP soil to 1.3% for the Charlton, New Hampshire, soil. If TNT were to bind with the soil organic matter at a level of 0.2% (w/w), the mass of carbon resulting from the TNT would only be 19 μg in a 2-g soil sample. This represents only 0.074% of the total carbon present in the sample, which is too small a difference to measure.

Incubation study 2: Unlimited contaminant source, maximum loading of soil

The objective of this experiment was to simulate a situation where an unlimited amount of contaminant material is available to the soil. At time zero, TNT was the only analyte in the system and was present solely in the aqueous phase (Table 6). By day 7, the analyte composition consisted of both TNT and the amino-DNTs, although it was primarily TNT, and it was distributed between the aqueous and soil phases. The total mass had decreased to 90% of the original mass of TNT (Table 7). By day 21 the percentage of amino-DNTs present increased, but the primary component of the system was still the TNT in the solution phase. The total mass had increased to 105% of the original mass and remained unchanged through day 56 (Table 6). The mass changes and redistribution of the analytes translated to concentrations of extractable TNT, 2ADNT, and 4ADNT of 25, 0.1, and 0.4 μg/g, respectively, after 14 days. At day 14, the concentration of the analytes in the aqueous phase was 95, 0.2, and 0.5 mg/L for TNT, 2ADNT, and 4ADNT, respectively. All of these concentrations remained relatively unchanged through 56 days.

The data plotted in Figure 9 showed definite trends in the change in concentration of TNT over time. The isotope data suggest a possible trend;

Table 7. Mass recovered from aqueous and soil phases in incubation study 2.

	Mass recovered (moles)					
	0	*7*	*14*	*21*	*28*	*56*
TNT in aqueous phase	4.36×10^{-5}	3.81×10^{-5}	4.39×10^{-5}	4.63×10^{-5}	4.59×10^{-5}	4.54×10^{-5}
2ADNT in aqueous phase	0.00	5.13×10^{-8}	1.15×10^{-7}	7.73×10^{-8}	6.54×10^{-8}	1.20×10^{-7}
4ADNT in aqueous phase	0.00	1.91×10^{-7}	4.03×10^{-7}	2.29×10^{-7}	1.56×10^{-7}	2.66×10^{-7}
TNT in soil	0.00	7.74×10^{-8}	3.72×10^{-7}	3.87×10^{-7}	3.51×10^{-7}	3.97×10^{-7}
2ADNT in soil	0.00	3.40×10^{-9}	1.99×10^{-9}	4.31×10^{-9}	1.45×10^{-9}	1.77×10^{-9}
4ADNT in soil	0.00	2.11×10^{-8}	1.43×10^{-8}	9.84×10^{-9}	6.76×10^{-9}	7.93×10^{-9}
Total moles recovered	4.36×10^{-5}	3.85×10^{-5}	4.49×10^{-5}	4.75×10^{-5}	4.65×10^{-5}	4.62×10^{-5}
Total moles added	4.47×10^{-5}	4.33×10^{-5}	4.55×10^{-5}	4.53×10^{-5}	4.44×10^{-5}	4.36×10^{-5}
Percent moles recovered	97.50%	88.91%	98.54%	104.76%	104.54%	105.90%

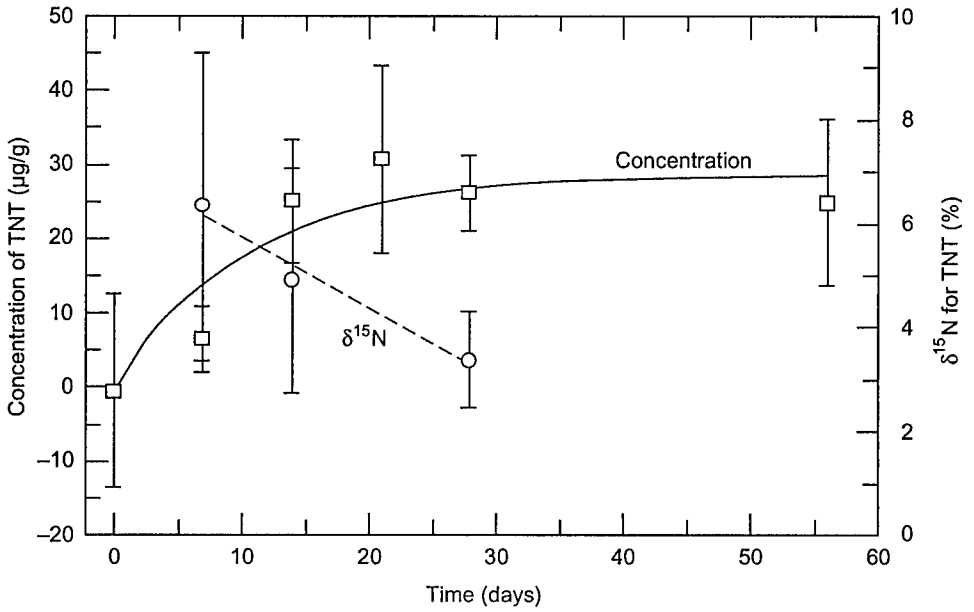

Figure 9. Concentration and $\delta^{15}N$ of extractable TNT over time for incubation study 2.

however, the precision of the measurements indicates that there is uncertainty whether or not this is real. Further investigation of this possible trend may be appropriate, based on results of the field studies conducted at LAAP discussed below.

Field studies

Isotope ratios across the contaminated plume at LAAP

The objective of this experiment was to determine if isotopic fractionation of the TNT in the groundwater was occurring as the TNT migrated through the soil. Groundwater samples were collected from six monitoring wells along four transects at LAAP in April 1998. The concentration of TNT in the groundwater near the source region of the plume ranged from 1870 up to 5110 µg/L. Concentrations along the leading edge ranged from 116 to 772 µg/L. Table 8 shows the data for the pairs of wells along the four transects.

A significant difference between the concentration of TNT in the center of the plume and that at the leading edge was evident for both the upper and lower terraces. Although no significant differences were found in the $\delta^{13}C$ values for TNT, the $\delta^{15}N$ values for TNT suggest possible trends. As predicted, the $\delta^{15}N$ values for TNT increased as the TNT moves away from the source area in the upper aquifer, but in the lower aquifer the opposite occurs. These results are not completely

Table 8. Concentration and stable isotope data for TNT extracted from LAAP groundwater.

	Plume	*Leading edge*
Transect 1		
Lower terrace	MW 141	MW 112
TNT conc. (µg/L)	2660	116
$\delta^{13}C$ (‰)	–20.3	–21.9
$\delta^{15}N$ (‰)	13.21	6.14
Transect 2		
Lower terrace	MW 141	MW 110
TNT conc. (µg/L)	2660	772
$\delta^{13}C$ (‰)	–20.3	–18.5
$\delta^{15}N$ (‰)	13.21	9.30
Transect 3		
Upper terrace	MW 140	MW 108
TNT conc. (µg/L)	1870	542
$\delta^{13}C$ (‰)	–20.9	–17.8
$\delta^{15}N$ (‰)	9.44	14.74
Transect 4		
Upper terrace	MW 085	MW 108
TNT conc. (µg/L)	5110	542
$\delta^{13}C$ (‰)	*	–17.8
$\delta^{15}N$ (‰)	11.36	14.74

*Data for this point were not available owing to a chromatographic interference.

Table 9. Concentration and $\delta^{15}N$ values for TNT extracted from LAAP groundwater collected in September 1998.

	Plume	*Leading edge*
Transect 1		
Lower terrace	MW 141	MW 112
TNT conc. (µg/L)	2870	75.1
$\delta^{15}N$ (‰)	13.3	17.3
Transect 2		
Lower terrace	MW 141	MW 110
TNT conc. (µg/L)	2870	1060
$\delta^{15}N$ (‰)	13.3	10.0
Transect 3		
Upper terrace	MW 140	MW 108
TNT conc. (µg/L)	1920	567
$\delta^{15}N$ (‰)	9.7	13.8
Transect 4		
Upper terrace	MW 085	MW 108
TNT conc. (µg/L)	5950	567
$\delta^{15}N$ (‰)	10.1	13.8

understood and the cause for these trends remains unclear. Further investigation through additional sampling and analysis may aide in clarifying these findings.

The wells at LAAP were sampled a second time in September 1998. Again, the TNT was extracted from the groundwater and the concentration and $\delta^{15}N$ values determined (Table 9). These data showed the same concentration gradients along each transect. The statistical analysis of the data (Table 10) showed a clear increasing trend in the $\delta^{15}N$ values for TNT, indicating that the TNT is being fractionated and that it has become isotopically heavier. These results strongly suggest that, as the TNT migrates through the soil, there is a process taking place that removes the lighter isotopic fraction of TNT from the groundwater. It was unclear why a decrease in the $\delta^{15}N$ values for TNT was seen along transect 2. Close examination of the site map (Fig. 1) showed that well 110, in the lower terrace, is directly beneath transect 4 in the upper terrace. Also, the $\delta^{15}N$ value for TNT from this sample is the same as that of the TNT found in the high concentration well along transect 4 (well 085). These data strongly suggest the possi-

Table 10. Statistical comparison of $\delta^{15}N$ values for TNT extracted from LAAP groundwater collected in September 1998.

	Mean	*SE*	F	*Pooled SE*	t
Transect 1					
Sample 141	13.26	0.956	6.32	1.83	–3.16
Sample 112	17.35	2.402			
Transect 2					
Sample 141	13.26	0.956	1.42	1.05	4.42
Sample 110	9.98	1.137			
Transect 3					
Sample 140	9.69	0.592	3.10	0.48	–12.22
Sample 108	13.85	0.336			
Transect 4					
Sample 085	10.10	0.689	4.20	0.54	–9.77
Sample 108	13.85	0.336			
Critical *t* values	95%	99%	99.9%		
t-values	2.78	4.60	8.60		
df = 3					
Critical *F* values	95%	99%			
F values	9.28	29.0			
df 3,3					

bility of communication between the groundwater of the upper and lower terrace. Such communication could explain the decrease in the $\delta^{15}N$ values for TNT along transect 2.

These results suggest the possibility of using $\delta^{15}N$ measurements of TNT from groundwater to monitor its fate or natural attenuation in the environment. Further studies would be required to understand how such a measurement could be used quantitatively. Factors that require investigation are the rate and magnitude of change in the $\delta^{15}N$ of TNT versus the concentration gradient of TNT across a plume, the contact time with the soil, and the mass of soil through which the TNT has passed. These parameters, as well as organic and moisture contents, would have to be assessed for several soil types.

Isotope ratios in soil at LAAP

TNT was detected in most of these samples at concentrations from 0.1 to 1.8 μg/g. Analysis of a subset of these samples indicated no difference between the $\delta^{13}C$ values for blank soil samples and those that were contaminated with TNT. The extractable TNT was also analyzed for $\delta^{15}N$; however, owing to the low concentration of TNT in the extracts, reliable measurements could not be obtained.

SUMMARY

The sensitivities of the $\delta^{13}C$ and $\delta^{15}N$ analyses were calculated to be 250 and 750 ng TNT, respectively. Below 4 ng TNT injected on the column, the $\delta^{13}C$ measurement was mass dependent. This threshold value translated to a soil concentration of 20 μg/g when extracting a 2-g sample with 10 mL of solvent. This is two orders of magnitude higher than the detection limits of the EPA standard method for explosives (USEPA 1994). The concentration of TNT in the soil samples collected at LAAP ranged from 0.1 to 1.8 μg/g. Even where the soil was in contact with an unlimited source of TNT for several weeks, the concentration of TNT adsorbed to the soil only reached 25 μg/g. Therefore, a change in the $\delta^{13}C$ value for the soil carbon resulting from the addition of TNT to the soil carbon cannot be measured because of the detection limitations of the analysis.

Sample preconcentration would improve detection, but may introduce greater measurement uncertainty. This may address the limitation for analytes in solution but not for soil. One of the key objectives was the ability to measure changes in the $\delta^{13}C$ value for the soil carbon as TNT becomes irreversibly bound to the soil organic matter. As discussed above, the change in mass of carbon in a soil sample resulting from TNT binding with organic matter in the soil is less than 0.1%. This is far too small a change to measure precisely.

The prediction that no changes in the ^{13}C stable isotope ratio would be detected for TNT in solution, even with large changes in concentration of TNT, was verified. Changes in the $\delta^{13}C$ for the soil carbon could not be detected as TNT became irreversibly bound to the soil organic matter. In incubation study 1, the concentration of TNT decreased by six-fold over a very short time, but no significant change in the $\delta^{13}C$ value for the soil was measured. In incubation study 2, the concentration of extractable TNT increased to 25 μg/g over 77 days, but no significant changes were measured in the $\delta^{13}C$ for soil carbon. Soil sample collected at LAAP showed no significant differences between samples from different locations or between samples from different depths at the same locations.

In a positive contrast to the ^{13}C data, the ^{15}N data suggested the feasibility of monitoring natural attenuation of TNT using $\delta^{15}N$ measurements. In two field experiments, increasing $\delta^{15}N$ values for TNT in the groundwater were observed as the TNT migrated through the soil. Data across three of the four transects at LAAP showed a significant increase in the $\delta^{15}N$ values for TNT with decreases in concentrations. These data are consistent with the reduction of the nitro groups to amino functionalities, followed by binding of the amino or di-amino product to the soil carbon (Pennington et al. 1998). Both steps in the process involve reactions of nitrogen groups; therefore, measuring $\delta^{15}N$ values is a very promising monitoring tool for natural attenuation of TNT and its transformation products in groundwater.

CONCLUSIONS

The objective of this work was to evaluate whether ^{13}C or ^{15}N stable isotope measurements of TNT or TNT-contaminated soil could be used as a monitoring technique for the natural attenuation of TNT. The ^{13}C data presented here clearly did not support the use of $\delta^{13}C$ measurements because changes in the $\delta^{13}C$ value for soil organic matter were too small to detect. The ^{15}N data, though, did show that changes in the $\delta^{15}N$ values for TNT in groundwater could be detected across a contamination plume and suggested the possibility of measurable changes being detected in

$\delta^{15}N$ values for TNT extracted from soil. These results indicate that further investigation into both the understanding of the fractionation processes and the methodology to measure them is warranted.

Two important pieces of information, though, surfaced out of these studies. First, the $\delta^{13}C$ and $\delta^{15}N$ values for TNT from multiple sources have been documented. These sources include analytical, technical, and military sources (from the U.S. and Europe). The second important point to note is that the sample preparation technology commonly used for extraction of TNT from either water or soil does not introduce an element of isotopic fractionation. These data may be used in future endeavors to determine the source of TNT contamination.

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